EXHIBIT 15

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Clinical Protocol

Phase I Study of Immunotherapy of Malignant Melanoma by Direct Gene Transfer

PRINCIPAL INVESTIGATOR: Evan M. Hersh, MD
Hematology/Oncology
Arizona Cancer Center
Tucson, AZ 85724

CO-PRINCIPAL INVESTIGATORS: Emmanuel Akporiaye, PhD
David Harris, PhD
Alison T. Stopeck, MD
Evan C. Unger, MD
James A. Warneke, MD

SPONSOR: Vical, Inc.
Steven A. Kradjian, Director
Regulatory Affairs
9373 Towne Centre Drive
Suite 100
San Diego, CA 92121

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Phase I Single and Multiple Dose Trial, with Dose Escalation, of HLA-B7 Plasmid DNA/DMRIE/DOPE Complex as an Immunotherapeutic Agent in Malignant Melanoma by Direct Gene Transfer

1.0 Background and Rationale

1.1 Overview

Cancer is a disease in which certain cells grow uncontrolled by the body's normal self-regulatory mechanisms. Traditional chemotherapy seeks to control cancer by killing rapidly dividing cells or by preventing cells from entering cell cycle and dividing. However, a number of non-malignant cells in the body such as bone marrow cells and intestinal epithelium cells are also rapidly dividing and hence are highly susceptible to the toxicity of chemotherapy. Doses sufficient to induce remission in the cancer cannot be administered without life-threatening side effects in 5–10% of the patients and the overall mortality from chemotherapy is 0.5%. A therapeutic approach that selectively kills tumor cells with high efficacy would theoretically be far superior to currently available therapies.

The goal of immunotherapy is to stimulate the immune system to recognize and kill cancer cells by modifying the tumor cells or modifying the host response by such mechanisms as expanding the lymphocytes that respond specifically to the antigens on the tumor cells. Immunotherapy has shown promise as an approach to the treatment of malignancy. Indeed, cancers such as melanoma, renal cell carcinoma and colon adenocarcinoma are responsive to modulation of immune function, because the immune system can be induced to recognize tumor-associated and tumor-specific antigens in these cells.

Over the last several decades, there have been many attempts to identify tumor-specific antigens that might be the targets for cytotoxic antibodies or cell-mediated immunity. There have been numerous attempts to develop vaccines and monoclonal antibodies directed at one or more preferentially expressed cell surface antigens in a variety of cancers. Overall, tumor vaccines using intact cells or extracts plus adjuvants have given about a 10–20% response rate. Other approaches to immunotherapy have involved the administration of non-specific immunomodulating agents such as Bacillus Calmette-Guerin (BCG), cytokines, and/or adoptive transfer of cytotoxic T cells, which have shown promise in animal models (1–6) and in man (7–10). More recently, molecular genetic interventions have been designed in an attempt to improve the efficacy of immunotherapy.

Nable and colleagues at the University of Michigan are investigating a novel molecular genetic intervention for human malignancy that enhances the immune response to tumors by in vivo gene transfer. This immunotherapeutic approach based on animal model work (11, 12) uses a gene encoding transplantation antigen, an allogeneic class I major histocompatibility complex (MHC) antigen, HLA-B7, introduced into human tumors in vivo by DNA/lipid complex transfection. The direct intratumoral injection approach is used. Expression of allogeneic MHC antigens on tumor cells stimulates immunity against both the transfected cells as well as previously unrecognized antigens present in unmodified tumor cells. The introduction of an allogeneic MHC gene directly into tumors in vivo has induced partial tumor regressions, as well as specific cytotoxic T cell responses to other antigens.

In a preliminary trial in humans with malignant melanoma Nabel treated 5 patients with malignant melanoma. Three patients received 3 treatments, totaling 0.87 μg of DNA intratumorally, and 3 patients (2 additional patients plus one of the original 3 patients) received cumulative dose of 2.58 μg of DNA via three treatments. No toxicity resulted from this form of treatment and there was no formation of anti DNA antibody or autoantibody. There was no plasmid DNA detectable in the blood by PCR following gene transfer (tested on days 3–7 post transfection at ~2 pg/ml sensitivity).

Evidence of gene transfer was found on biopsy of the injected tumor. The biopsy samples were analyzed for plasmid DNA, mRNA coding for HLA-B7 and the expression of HLA-B7 protein. In 4 of the 5 patients, plasmid DNA and HLA-B7 mRNA were detected within the treated nodules by PCR. HLA-B7 expression was confirmed in all treated nodules by immunohistochemical staining with a monoclonal antibody to the gene product. Two patients, where cell lines were established from the tumor, showed an immune response by lysing autologous tumor cells. One of the 5 patients had a partial remission which involved cutaneous and visceral disease (12A). These data suggest that tumor cells modified with the HLA-B7 gene not only stimulate CTLs and potentially other immune system cells to recognize tumors expressing HLA-B7, but they may also provide a stimulus to immune cells to eliminate tumor cells at other sites which express tumor associated antigens in association with the patient's own HLA antigens.

Several improvements that may increase the convenience, safety and efficacy of the procedure have been introduced since the original Nabel studies including:

- an improved cationic lipid formulation, DMRIE/DOPE*;
- DNA plasmid construction to optimize expression

The efficacy of transfection was improved for the following reasons. Briefly, a new formulation of cationic lipids has been described recently by Dr. Phillip Felgner (Vical) in which a different cationic lipid, 1,2-dimyristyloxypropyl-3-dimethyl-hydroxyethyl ammonium bromide (DMRIE), is utilized with dioleoyl phosphatidylethanolamine (DOPE). This has two properties which make it more suitable for these studies. First, it shows up to 10-fold improved transfection efficiency in vitro compared to the formulation previously used by Nabel. More

*DMRIE, 1,2-dimyristyloxypropyl-3-dimethyl-hydroxyethyl ammonium; DOPE, dioleoyl phosphatidylethanolamine bromide.

importantly, this formulation does not aggregate at high concentrations. This characteristic thus allows higher absolute concentrations of DNA and lipid complex to be introduced into experimental animals without toxicity. Because of these properties, it now becomes possible to introduce 100–1000 times more DNA which could allow the study of an expanded dose response gene expression in vivo.

The vector improvements are divided into two categories for this proposal. In the first case expression of the HLA-B7 vector has been improved by the addition of a consensus translation initiation sequence and removal of an intron. In addition, the inclusion of the β-2 microglobulin gene, with which class I MHC genes normally associate, allows synthesis of the complete histocompatibility molecule, which is composed of these two chains. Ordinarily, these two gene products are co-transported to the cell surface. This is important because some human melanoma cells do not express endogenous β-2 microglobulin, thus limiting their ability to stably express class I on the cell surface. It has been found that the inclusion of the β-2 microglobulin gene on the same plasmid allows for the expression in these otherwise resistant cells and improve expression in other cells, thus overcoming a potential mechanism of resistance. These modifications have been incorporated in the study drug to be used in this submission. The study drug is identical to the study drug fully characterized in Dr. Nabel's RAC submission of June 7, 1993, which was unanimously allowed. This study will investigate the administration of the study drug in malignant melanoma.

1.2 Background

1.2.1 Direct Gene Transfer and Modulation of the Immune System

The utilization of catheter-based gene delivery in vivo provided a model system for the introduction of recombinant gene-specific sites in vivo. Early studies focused on the demonstration that specific reporter genes could be expressed in vivo (13,14). Subsequent studies were designed to determine whether specific biologic responses could be induced at sites of recombinant gene transfer. To address this question, a highly immunogenic molecule, a foreign major histocompatibility complex (MHC), was used to elicit an immune response in the iliofemoral artery using a porcine model. The human HLA-B7 gene was introduced using direct gene transfer with a retroviral vector or DNA lipid complex (12). With either delivery system, expression of the recombinant HLA-B7 gene product could be demonstrated at specific sites within the vessel wall. More importantly, the expression of this foreign histocompatibility antigen induced an immunologic response at the sites of genetic modification. This response included a granulomatous mononuclear cell infiltrate beginning 10 days after introduction of the recombinant gene. This response resolved by 75 days after gene transfer; however, a specific cytolytic T cell response against the HLA-B7 molecule was persistent. This study demonstrated that a specific immunologic response could be induced by the introduction of a foreign recombinant gene at a specific site in vivo. Moreover, this provided one of the first indications that direct gene transfer of specific recombinant genes could elicit an immune response to the product of that gene in vivo (12).

These studies suggested that the introduction of the appropriate recombinant genes could be used to stimulate the immune system to recognize its product in vivo. In addition, this approach provided a general method for the induction of a specific site in vivo. To determine whether direct gene transfer might be appropriate for the treatment of disease, a murine model of malignancy was developed. Direct gene transfer of an allogeneic histocompatibility complex gene into a murine tumor elicits an immune response not only to the foreign MHC gene but also to previously unrecognized tumor-associated antigens. These immune responses are T cell-dependent, and these tumor-associated proteins are recognized within the context of the self major histocompatibility complex. In animals presensitized to a specific MHC haplotype, direct gene transfer into established tumors could attenuate tumor growth or, in some cases, lead to complete tumor regression (11). These studies demonstrate that direct gene transfer of foreign MHC genes into tumors have potentially therapeutic effects that may be appropriate for the treatment of malignancy.

1.2.2 Immunotherapy of Malignancy

In some instances, the immune system appears to contribute to the surveillance and destruction of neoplastic cells, either by mobilization of cellular and humoral immune efforts. Cellular mediators of anti-tumor activity include MHC-restricted cytotoxic T cells, natural killer (NK) cells (15,16) and lymphokine-activated killer (LAK) cells (17). Cytolytic T cells which infiltrate tumors have been isolated and characterized (18). These tumor infiltrating lymphocytes (TIL) selectively lyse cells of the tumor from which they were derived (3,19). Macrophages can also kill neoplastic cells through antibody-dependent mechanisms (20,21), or by activation induced by substances such as BCG (22).

Cytokines can also participate in the anti-tumor response, either by a direct action on cell growth or by activating cellular immunity. The cytostatic effect of tumor necrosis factor-α (TNF-α) (23) and lymphotoxin (24) can result in neoplastic cell death. Interferon-γ (IFN-γ) markedly increases class I MHC cell surface expression (25,26) and synergizes with TNF-α in producing this effect (27). Colony stimulating factors such as G-CSF and GM-CSF activate neutrophils and macrophages to lyse tumor cells directly (28), and interleukin-2 (IL-2) activates Leu-19$^+$ NK cells to generate lymphokine activated killer cells (LAK) capable of lysing autologous, syngeneic or allogeneic tumor cells but not normal cells (17,29,30). The LAK cells lyse tumor cells without preimmunization or MHC restriction (31). Interleukin-4 (IL-4) also generates LAK cells and acts synergistically with IL-2 in the generation of tumor specific killer cells (32).

Since most malignancies arise in immunocompetent hosts, it is likely that tumor cells have evolved mechanism to escape host defenses, perhaps through evolution of successively less immunogenic clones (33). Deficient expression of class I MHC molecules limits the ability of tumor cells to present antigens to cytotoxic T cells. Freshly isolated cells from naturally occurring tumors frequently lack class I MHC antigen completely or show decreased expression (34–38). Reduced class I MHC expression could also facilitate growth of these tumors when transplanted into syngeneic recipients. Several tumor cell lines

which exhibit low levels of class I MHC proteins become less oncogenic when expression vectors encoding the relevant class I MHC antigen are introduced into them (39–43). In some experiments, tumor cells which express a class I MHC gene confer immunity in naive recipients against the parental tumor (40,41). The absolute level of class I MHC expression however, is not the only factor which influences the tumorigenicity or immunogenicity of tumor cells. In one study, mouse mammary adenocarcinoma cells, treated with 5-azacytidine and selected for elevated levels of class I MHC expression did not display altered tumorigenicity compared to the parent line (44).

The immune response to tumor cells can be stimulated by systemic administration of IL-2 (45), or IL-2 with LAK cells (46,47). Clinical trials using tumor infiltrating lymphocytes are also in progress (48). Recently, several studies have examined the tumor suppressive effect of lymphokine production by genetically altered tumor cells. The introduction of tumor cells transfected with an IL-2 expression vector into syngeneic mice stimulated an MHC class I restricted cytolytic T lymphocyte response which protected against subsequent rechallenge with the parental tumor cell line (49). Expression of IL-4 by plasmacytoma or mammary adenocarcinoma cells induced a potent antitumor effect mediated by infiltration of eosinophils and macrophages (50). These studies demonstrate that cytokines, expressed at high local concentrations, are effective anti-tumor agents.

Nabel and co-workers have previously proposed an alternative approach to stimulate an anti-tumor response, through the introducion of an allogeneic class I MHC gene into established human tumors. The antigenicity of tumor cells has been altered previously by the expression of viral antigens through infection of tumor cells (51–55), or expression of allogeneic antigens introduced by somatic cell hybridization (56,57). Allogeneic class I MHC genes have been introduced into tumor cells by transfection and subsequent selection in vitro. These experiments have produced some conflicting results. In one case, transfection of an allogeneic class I MHC gene (H-2L^d) into an H-2^b tumor resulted in immunologic rejection of the transduced cells and also produced transplantation resistance against the parent tumor cells (58). In another instance, transfection of H-2^b melanoma cells with the H-2D^d gene did not lead to rejection (59), however increased differential expression of H-2D products relative to H-2K may have affected the metastatic potential and immunogenicity of tumor cells (60). The effects of allogeneic H-2K gene expression in tumor cells was examined in another study (61). Several subclones which were selected in vitro and expressed an allogeneic gene were rejected in mice syngeneic for the parental tumor line, however, other subclones did not differ from the parental, untransduced line in generating tumors. This finding suggests that clone-to-clone variation in in-vivo growth and tumorigenic capacity may result in other modifications of cells caused by transfection or the subcloning procedure, which affects their tumorigenicity. These types of clonal differences would likely be minimized by transducing a population of cells directly in vivo.

Because the H-2K class I MHC antigen is strongly expressed on most tissues and can mediate an allogeneic rejection response, he chose it in our animal model studies designed to enhance the immunogenicity of tumors in vivo. These studies extended previous efforts to modify tumor cells by developing a system for the direct introduction of genes into tumors by in vivo infection using retroviral vectors or by DNA lipid complex mediated transfection. This technology can also be used to deliver specific recombinant cytokines into the tumor microcirculation and to understand the immunologic basis for tumor rejection in vivo.

2.0 Objectives

2.1 To determine safety and toxicity of direct intralesional injection of increasing amounts of a DNA/lipid mixture: VCL 1005 (HLA-B7/DMRIE/DOPE) into patients with metastatic malignant melanoma. Escalating treatment regimens will be used and tumor growth evaluated.

2.2 To measure the cytotoxic T cell (CTL) activity directed toward antigens on tumor cells other than HLA-B7 by measuring CTL activity against autologous tumor cells when available.

2.3 To measure humoral and cellular immune responses to HLA-B7.

2.4 To confirm expression in vivo of the HLA-B7 gene in the tumor cells.

2.5 To characterize the clinical response to escalating doses of the study drug by assessing the size of the injected tumor and of all other tumor masses that may be present and to define the response in terms of the standard oncologic criteria for complete remission, partial remission, stable disease and progression.

3.0 Study Design

This is a Phase I open-label multi-center study in which fifteen patients per center will be enrolled for direct injections with lipid-formulated HLA-B7 plasmids. Each center will enter patients with one tumor type. The Arizona Cancer Center will enroll patients with melanoma. There will be two study arms, single injections (arm 1) and multiple injections (arm 2). Eligible patients will have one solid tumor nodule injected with the study drug (in the single-dose phase of the study), whereas patients enrolled in the repeated-dose phase of the study will have either two or three nodules injected.

Increasing doses of the study drug will be administered in the single-dose phase of the study. Three dosage groups with three patients each will be studied sequentially with at least one month of observation post-injection on the first patient in the dosage group prior to advancing to the next higher dosage.

Initially, arm I patients will receive a single injection of 10 μg of the study drug. Following completion of the first dosage group of three patients, a second single dosage group of three patients will be treated at the second level and the repeated dose arm of the study will be initiated. Group II will receive a five-fold higher concentration of study drug (50 μg). Following completion of the second single dosage group of three patients, a third group of three patients will be treated. Group III will receive a 5-fold higher dose (250 μg) than Group II patients.

The dose will be raised to the next higher level if none of the three patients exhibit any toxicities of grade III or higher. If a

patient displays toxicities of grade III or higher, then that dose will be repeated on three additional patients. This phase of the study is summarized in the following table:

ARM 1

Dosage group	*No. of patients per group*	*Dose per treatment*	*Total no. of treatments per patient*
I	3	10 μg	1
II	3	50 μg	1
III	3	250 μg	1

Patients in arm 2 will receive repeated injections of 10 μg of the study drug. The dose schedule given below will be carried out and second and third doses will be administered to each subject in whom no grade III or greater toxicities are observed. Second and third doses will be administered in different tumors than the first dose if such are available. If not they will be administered in the same tumor nodule.

ARM 2

Dosage group	*No. of patients per group*	*Dose per treatment*	*Total no. of treatments per patient*	*Days between treatments*
I	3	10 μg	2	15
II	3	10 μg	3	15,43

Arm 1 patients will be followed for 8 weeks with a tumor staging at that time. Arm 2 patients will be followed for 10 weeks with a tumor staging at that time. See Appendix 1 for the schedule of events.

Criteria for re-treatment: Patients with a partial or complete response at 4 weeks after the last injection of their initial course may receive an additional course of treatment identical to the first treatment, i.e., on Schedule A, at 4 weeks a responding patient may receive a second injection at the same dose initially given; on Schedule B, 4 weeks after two injections (B-1) and 4 weeks after three injections (B-2), responding patients may receive a second course identical to their first course.

4.0 Patient Eligibility

Patients will be carefully selected based on their past medical history and present status. The Investigator and the patient will make a joint decision regarding the appropriate treatment with conventional therapy. Patients will be selected who have failed on conventional therapy or for whom conventional therapy is not indicated.

4.1 Inclusion Criteria

4.1.1 Diagnosis: Histologically confirmed metastasis of malignant melanoma
Histologic confirmation may be by needle biopsy at the time of intratumoral injection.

4.1.2 Patients must have at least one metastatic lesion measurable in two dimensions and at least 1 cm in longest diameter.

4.1.3 Patients must have had either prior standard therapies for their disease and have become unresponsive to them or have made the decision that other therapy would not be of any benefit.

4.1.4 Patients must be adults 18 years of age or older.

4.1.5 Patients must be HLA-B7 negative.

4.1.6 Patients must have adequate bone marrow reserve: WBC $> 3000/mm^3$, platelets $> 100{,}000/mm^3$, hemoglobin > 9.0 gm and adequate renal and liver function, creatinine ≤ 2.0 and bilirubin < 2.0 mg/DL.

4.1.7 Patients must have a baseline Karnofsky Performance Status (KPS) socre of at least 70. (Appendix 2)

4.1.8 Estimated life expectancy of at least 12 weeks.

4.1.9 Patients must be anti-HIV antibody negative.

4.1.10 Investigator must have obtained signed informed consent from patient.

4.1.11 If patient is female, she must either not be of child-bearing potential by reason of surgery, radiation or menopause or, if of child-bearing potential, using an approved method of contraception.

4.1.12 Patients must be immunocompetent by having a PHA lymphocyte response in the normal range.

4.2 Exclusion Criteria

4.2.1 Patients with an active autoimmune disease.

4.2.2 Patients with active hepatitis (chronic or acute) or HB_s Ag positive, and patients who are HIV seropositive

4.2.3 Patients with any active infection requiring parenteral antibiotics.

4.2.4 Radiation, chemotherapy, steroidal therapy within the past three weeks. Surgery within the past 2 weeks.

4.2.5 Patients receiving concurrent anticancer drug therapy, any immunosuppressive drugs and any other experimental therapy.

4.2.6 Immunoincompetence as defined above in 4.1.12.

4.2.7 Patients with diabetes mellitus who are not controlled by medical treatment.

4.2.8 Patients with significant psychiatric disorders that would make compliance to the Protocol difficult.

5.0 Study Drug

The study drug (VCL-1005) will be supplied as three sterile vials containing (i) HLA-B7 plasmid DNA, (ii) DMRIE/DOPE lipid mixture and (iii) lactated Ringer's. All components will be stable for at least eight weeks under recommended storage

conditions (DNA −20°C, DMRIE/DOPE 4°C). The materials will be supplied by Vical Inc.

5.1 VCL 1005 (HLA-B7 Plasmid DNA)/ DMRIE/DOPE:

This study drug is composed of plasmid DNA coding for the complete human MHC HLA-B7 formulated with the cationic lipid mixture DMRIE/DOPE (lipid complex formulation). The DNA concentration is 1.0 mg/ml (see Investigator's brochure for complete details of product characteristics and preparation).

5.2 Lactated Ringer's:

Readily available at the site.

6.0 Study Drug Administration and Toxicities to Be Monitored

DNA/lipid-complexes are prepared immediately prior to administration. DNA is supplied in 1.0 mg/ml concentration in 400 μl lactated Ringer's solution. Lipid (DMRIE/DOPE) is supplied as a dried film. Each vial contains 77 μg DMRIE and 90 μg DOPE. Each vial is reconstituted with 400 μl lactated Ringer's solutions by vortexing until homogeneous. The contents of the lipid vial is transferred into the DNA vial and mixed well by repeated inversion. The final concentration of the HLA-B7 plasmid DNA is 500 μg/ml. The amount injected into each tumor will be based on the algorithm outlined below. The doses of 10, 50 and 250 μg, will be prepared and formulated as dilutions with lactated Ringer's. After injection of the drug and with the needle still in place the dead space will be flushed with 0.25–0.50 ml of lactated Ringer's.

Tumor diam. (cm)	*Volume of injection (cc)*
1.0–1.5	0.5
1.6–2.0	2.0
2.1–3.0	3.0
3.1–x	4.0

Tumor lesions will be selected for treatment if they are accessible to intratumor administration by direct needle injection. These metastatic lesions will be located at any accessible site such as skin, nodes, lung, liver, spleen. If necessary, the study drug will be injected with the aid of sonographic or CAT scan visualization of the metastasis. Prior to injection following placement of the needle, gentle aspiration will be applied to the syringe to ensure that no material is injected intravenously. Vital signs will be measured every 15 minutes prior to, during, and after the injection for at least two hours or until the patient is stable. If the systolic blood pressure drops below 80 mm Hg, the injection will be terminated immediately and the patient will be closely monitored or treated appropriately until blood pressure is normalized.

Within 1 hour after the injection, a blood sample will be obtained to check serum enzymes, blood chemistries and cell counts, and to analyze by PCR for the presence of HLA-B7 plasmid DNA in the peripheral blood. Every patient will be checked at 48 hours and another blood collection will be drawn as described in Section 7 below. If any abnormalities appear, the patient will be closely observed. All toxicities will be graded according to the World Health Organization (WHO) recommendations (see Appendix 3).

7.0 Safety and Efficacy

No more than two weeks prior to entry into the study, and several times during the study according to the Schedule of Events chart (Appendix 1), all patients will have the following studies performed to assess their disease status:

7.1 Physical exam and medical history including height, weight, performance status and tumor measurements (tumor staging and by physical exam [when accessible]).
7.2 Chest x-ray.
7.3 EKG.
7.4 CBC, platelet count, differential, PT, PTT.
7.5 Blood chemistries: creatinine, BUN, bilirubin, alkaline phosphatase, LDH, SGOT, SGPT, phosphorus, uric acid, calcium, total protein, albumin, glucose.
7.6 Merieux Multitest skin tests.
7.7 Lymphocyte Proliferative Response to PHA.
7.8 Pregnancy test for women.
7.9 HIV antibody test.
7.10 Hepatitis screen.
7.11 Tumor biopsies (through an 18–20 g needle).
biopsy requirements:
minimum: 1 core (1–2 cm × 1 mm) snap frozen LN2 cryovial
additional: 2 cores, one on wet ice, one snap frozen
3 cores, one on wet ice, two snap frozen
The ability to obtain more than one core will be at the judgement of the investigator.
7.12 CT/MRI/Ultrasound (if relevant) for baseline and to evaluate status of disease.
7.13 Special Tests: Blood will be drawn for serum to be analyzed by PCR for the presence of the plasmid and for the presence of antibody to HLA-B7 and to autologous tumor cells (when available). PBLs will be isolated and analyzed for their ability to lyse HLA-B7 modified autologous EBV-transformed lines and to lyse autologous tumor cells (when available).
7.14 Blood draws during this study will be limited to a maximum of 50 ml each.

8.0 Adverse Events and Removal from Study

8.1 Patients will be monitored and questioned at every visit (see Schedule of Events) regarding the occurrence and nature of any adverse experiences. An event is any change in the physiological or psychological state other than the primary condition that qualifies the patient for this study.
8.2 The Investigator must report to Mr. Steven Kradjian (Ph 619-453-9900, available 24 hrs.) upon occurrence of any life-threatening events (Grade IV) that may be attributable to administration of the study drug, all fatal events, or the first occurrence of any previously un-

known clinical event (regardless of Grade). A written report is to follow within 3 working days to:

Steven A. Kradjian
Vical Inc.
9373 Towne Centre Drive, Suite 100
San Diego, CA 92121

8.3 Patients will be taken off study immediately if:

8.3.1 Unacceptable toxicity (Grade III or IV) develops and is not easily corrected (refer to WHO toxicity sheet).

8.3.2 Development of Progressive Disease (see definition 9.2.4) requiring the institution of alternative treatments such as radiation, surgery or other drug therapy.

8.3.3 If the Investigator believes that the patient's best interest requires a change of therapy.

8.3.4 At the patient's or guardian's request.

9.0 Efficacy Criteria

9.1 Pharmacologic/Immunologic Studies

As part of the measurement of the efficacy in this study, successful gene transfer and expression will be evaluated by molecular genetic and immunological analyses. Several independent techniques will be used to evaluate the presence and expression of the recombinant gene in vivo.

9.1.1 Monoclonal antibodies to HLA-B7 and immunochemistry techniques will be used to detect the presence of the recombinant gene product in biopsied samples. Fluorescence staining of freshly dispersed cells will also be evaluated when it is possible to remove sufficient tumor to make the cell suspensions.

9.1.2 Successful gene transfer will be assessed by the presence of DNA from the HLA-B7 gene as detected by PCR amplification of DNA from cells obtained by biopsy of the treated sites on various days after the injection of the study drug (see Schema). Genomic DNA will be isolated by standard methods and a portion of the HLA-B7 gene will be amplified and sequenced.

9.1.3 Circulating antibodies to HLA-B7 will be measured by immunoassay. The presence of antibody will be evaluated by FACS analysis of a matched pair of HLA-B7-positive and -negative cell lines. Circulating antibody to autologous tumor will also be measured by immunoassay when such cells are available from prior biopsies.

9.1.4 Autologous peripheral blood lymphocytes will be immortalized by EBV transformation and subjected to in vitro gene transfer with the DNA/liposomal complex. These autologous cells expressing the HLA-B7 gene will be used to assess the specificity of patients' immune responses to the HLA-B7 antigen. Direct gene transfer and expression of the HLA-B7 gene is expected to sensitize the patient to HLA-B7 and lead to the generation of an immune response to this antigen. Limiting dilution analysis (LDA) will be done to evaluate alterations in the frequency in HLA-B7-specific helper and cytolytic T cells in the peripheral blood following direct gene transfer.

9.1.5 Peripheral blood lymphocytes will be isolated and cyro-preserved prior to, and at 2- to 4-week intervals following, the initial treatment. At the completion of treatment, PBL samples from each time point will be simultaneously evaluated for responsiveness to HLA-B7 by culturing PBLs, under LDA conditions, with autologous EBV-transformed cells transfected with the HLA-B7 gene. Antigen specific elaboration of IL-2 or generation of CTLs to HLA-B7-positive target cells will be the indices evaluated in these studies. The presence of antibody will be evaluated by FACS analysis of a matched pair of HLA-B7-positive and -negative cell lines.

9.1.6 In selected instances, lymphocytes will be isolated directly from the tumor, expanded in tissue culture, and analyzed for cytolytic activity. Tumor biopsies at 7 and 14 days after treatment will be analyzed by immunochemistry. An attempt will be made to isolate and expand draining lymph node T cells or TIL cells to test their cytolytic activity.

9.1.7 When possible, autologous cell lines will be derived from a pre-treatment tumor specimen and used in a ^{51}Cr-release assay with peripheral blood lymphocytes.

9.1.8 Finally, every attempt will be made to excise tumor tissue prior to treatment for diagnosis, immunochemistry, cyro-preservation and to evaluate delayed-type hypersensitivity reactions to the tumor before and after treatment.

9.1.9 Evidence of efficacy will include:

A. Introduction of DNA as induced by PCR.
B. Expression of HLA-B7 by immunohistochemistry.
C. Antibody response to HLA-B7.
D. Antibody response to autologous tumor.
E. CTL response to HLA-B7.
F. CTL response to autologous tumor.

9.2 Clinical Response Criteria

All patients who receive at least one dose of study drug will be analyzed for efficacy and safety. Standard oncologic criteria will be applied to determine whether or not a patient responds to the study drug. All tumor measurements must be recorded in centimeters and should consist of the longest diameter and the perpendicular diameter at the widest portion of the tumor. All the following determinations of tumor response should be made comparing current total tumor size to pre-treatment total tumor size.

9.2.1 Complete Tumor Response

Disappearance of all clinical evidence of active tumor for a minimum of four weeks. The patients must be free of all symptoms of cancer.

9.2.2 Partial Tumor Response

Fifty percent (50%) or greater decrease in the sum of the products of all diameters of measurable lesions. These reductions in tumor size must endure for a minimum of four weeks. No simultaneous increase in the size of any lesion or appearance of new lesions may occur. The appropriate diagnostic tests used to demonstrate the response must be repeated four weeks after initial observation in order to document this duration.

9.2.3 Stable Disease

Less than 50% decrease in the sum of the products of all diameters of measurable lesions, or an increase in the tumor mass less than 25% in the absence of the development of new lesions.

9.2.4 Progressive Disease

Tumor progression will be defined if one or more of the following criteria are met:

- Appearance of any new lesion(s).
- Increase in tumor size of ≥25% in the sum of the products of all diameters of measurable lesions.
- Significant clinical deterioration that cannot be attributed to treatment or other medical conditions and is assumed to be related to increased tumor burden.
- Worsening of tumor-related symptoms clinically significant by physician.

10.0 Informed Consent and IRB Approval

The Investigator at each site will be responsible for and shall prepare a written Informed Consent Document. The Protocol and the Informed Consent Document must be provided to his IRB. The IRB must approve the Protocol and Informed Consent Document and agree to monitor the conduct of the study and periodically review its progress at no less than one year intervals. Before the study begins, the Investigator will provide the Sponsor with documented evidence, i.e., a letter from the IRB or a copy of the IRB minutes, of:

- Protocol and Informed Consent approval.
- The IRB agreement to monitor the conduct of the study and review it periodically.

10.1 Updates to the Informed Consent Documents

The Sponsor will notify the Investigator should significant new findings develop during the course of the study that may relate to the patient's willingness to continue participation. It is the Investigator's responsibility to:

- Notify the local IRB.
- Update the Informed Consent Document.
- Notify each patient accordingly.
- Each patient's record must contain a signed copy of the updated Informed Consent Document.
- Send the Sponsor a copy of each patient's signed updated Informed Consent Document.

11.0 Statistic Considerations

Descriptive statistics will be performed due to the small number of patients.

12.0 References

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APPENDIX 1. SCHEDULE OF EVENTS

	Pre-study	*During treatment*						
		1	3	15	29	6	8	[5]10
Physical exam	X	X		X	X	X	X	X
History[1]	X			X	X	X	X	X
Performance Status	X						X	X
Chemistry Survey[2]	X	X	X	X	X	X	X	X
Vital signs	X	X	X	X	X	X	X	X
Weight	X	X	X	X	X	X	X	X
CBC,Diff,Platelet	X	X	X	X	X	X	X	X
PT,PTT	X							
EKG	X							
CXR	X							
Hb_sAg, HIV	X							
Brain CT OR MRI[3]	X							
Assess for adverse events status	X	X	X	X	X	X	X	X
Tumor biopsy[4]	X			X	X	(X)	(X)	
Assay for HLA-B7 cytotoxic T-cells	X			X	X	X		X
Assay for HLA-B7 antibody	X			X	X	X		X
Pregnancy test(if relevant)	X							
Anti-DNA antibodies ds DNA ss DNA	X				X	X		X
Quantitate size of lesion including tumor imaging by X-ray or scans (tumor staging)	X			[6]X		X		X

1. Follow up history is limited and focused on present illness and treatment effects only.
2. Routine laboratory test include uric acid, calcium, phosphate, SGOT, SGPT, alkaline phosphatase, LDH, bilirubin, BUN, creatinine, total protein, glucose.
3. If clinically indicated only.
4. To be performed on nodules 1 week prior to treatment. Nodules will be ≥1 cm. Follow up needle biopsies on D15 and 29 in arm 1 and on D15 and either week 6 or 8 in arm 2.
5. Week 10 studies only on arm 2 patients.
6. For lesions detectable by physical exam only.

APPENDIX 2. KARNOFSKY PERFORMANCE SCALE

Activity status	*Point*	*Description*
Normal Activity	100	Normal, with no complaints or evidence of disease
	90	Able to carry on normal activity but with minor signs or symptoms of disease
	80	Normal activity but requiring effort; signs and symptoms of disease more prominent
Self-Care	70	Able to care for self, but unable to work or carry on other normal activities
	60	Able to care for most needs but requires occasional assistance
	50	Considerable assistance required, along with frequent medical care; some self-care still possible
Incapacitated	40	Disabled and requiring special care and assistance
	30	Severely disabled; hospitalization required but death from disease not imminent
	20	Extremely ill, supportive treatment, hospitalized care required
	10	Imminent death
	0	Dead

APPENDIX 3. WHO RECOMMENDATIONS FOR GRADING OF ACUTE AND SUB-ACUTE TOXIC EFFECTS

	Grade 0	*Grade 1*	*Grade 2*	*Grade 3*	*Grade 4*
Hematological (Adults)					
Hemoglobin	≥11.0 g/100 ml ≥110 g/l ≥6.8 mmol/l	9.5–10.9 g/100 ml 95–109 g/l 5.6–6.7 mmol/l	8.0–8.4 g/100 ml 80–94 g/l 4.95–5.8 mmol/l	6.5–7.9 g/100 ml 65–79 g/l 4.0–4.9 mmol/l	<6.5 g/100 ml <65 g/l <4.0 mmol/l
Leukocytes ($1000/mm^3$)	≥4.0	3.0–3.9	2.0–2.9	1.0–1.9	1.0
Granulocytes ($1000/mm^3$)	≥2.0	1.5–1.9	1.0–1.4	0.5–0.9	<0.5
Platelets ($1000/mm^3$)	>100	75–99	50–74	25–49	<25
Hemorrhage	None	Petochiae	Mild blood loss	Gross blood loss	Debilitating blood loss
Methemoglobin[a]	0–2.0%	2.1–10.0%	10.1–20.0%	20.1–30.0%	>30%
Gastrointestinal					
Bilirubin	≤1.25 × N[b]	1.26–2.5 × N[b]	2.6–5 × N[b]	5.1–10 × N[b]	>10 × N[b]
Transaminases (SGOT/SGPT)	≤1.25 × N[b]	1.25–2.5 × N[b]	2.6–5 × N[b]	5.1–10 × N[b]	>10 × N[b]
Alkaline phosphatase	≤1.25 × N[b]	1.26–2.5 × N[b]	2.6–5 × N[b]	5.1–10 × N[b]	>10 × N[b]
Oral	No change	Soreness/erythema	Erythema, ulcers; can eat solids	Ulcers; requires liquid diet only	Alimentation not possible
Nausea/vomiting	None	Nausea	Transient vomiting	Vomiting requiring therapy	Intractable vomiting
Diarrhea	None	Transient, <2 days	Tolerable, but >2 days	Intolerable, requiring therapy	Hemorrhagic dehydration
Renal					
Blood urea nitrogen or Blood urea creatinine	≤1.25 × N[a]	1.26–2.5 × N[a] 1+	2.6–5 × N[a] 2–3+	5–10 × N[a] 4+	>10 × N[a]
Proteinura	No change	<0.8 g% <3 g/l	0.3–1.0% <3–10 g/l	>1.0 g% >10 g/l	Nephrotic syndrome
Hematuria	No change	Microscopic	Gross	Gross + clots	Obstructive uropathy
Pulmonary	No change	Mild symptoms	Exterional dyapnea	Dyapnea at rest	Complete bed rest required
Fever with drug	None	Fever <38°C	Fever 38°C–40°C	Fever >40°C	Fever with hypotension
Allergic	No change	Erythema	Dry desquamation, vesiculation, prurieus	Moist desquamation, ulceration	Exfoliative dermatitis; necrosis requiring surgical intervention
Hair	No change	Minimal hair loss	Moderate, patchy alopecia	Complete alopecia, but reversible	Non-reversible alopecia
Infection (specify site)	None	Minor infection	Moderate infection	Major infection	Major infection with hypotension
Cardiac Rhythm	No change	Sinus tachycardia >110 at rest	Unifocal PVC, atrial arrhythmia	Multifocal PVC	Ventricular tachycardia
Cardiac Function	No change	Asymptomatic, but abnormal cardiac sign	Transient symptomatic dysfunction; no therapy required	Symptomatic dysfunction responsive to therapy	Symptomatic dysfunction non-responsive to therapy
Pericarditis	No change	Asymptomatic effusion	Symptomatic; no tap required	Tamponade; tap required	Tamponade; surgery required

(Continued)

APPENDIX 3. *Continued*

	Grade 0	*Grade 1*	*Grade 2*	*Grade 3*	*Grade 4*
Neurotoxicity					
State of consciousness	Alert	Transient lethargy	Somnolence <50% of waking hours	Somnolence >50% of waking hours	Coma
Peripheral	None	Paresthesias and/or decreased tendon reflexes	Severe paresthesias and/or mild weakness	Intolerable paresthesias and/or marked motor loss	Paralysis
Constipation[c]	None	Mild	Moderate	Abdominal distension	Distension and vomiting
Pain[d]	None	Mild	Moderate	Severe	Intractable

[a] Any degree of methemoglobinemia associated with what is felt to be clinically significant pulmonary symptoms or cyanosis will be considered at least grade 3.

[b] Upper limit of normal value of population under study.

[c] This does not include constipation resultant from narcotics.

[d] Only treatment-related pain is considered, *not* disease-related pain. The use of the narcotics may be helpful in grading pain, depending upon the tolerance level of the patient.

SUBJECTS' CONSENT FORM
TITLE: Phase I Study of Immunotherapy
of Malignant Melanoma by
Direct Gene Transfer

I AM BEING ASKED TO READ THE FOLLOWING MATERIAL TO ENSURE THAT I AM INFORMED OF THE NATURE OF THIS RESEARCH STUDY AND OF HOW I WILL PARTICIPATE IN IT, IF I CONSENT TO DO SO. SIGNING THIS FORM WILL INDICATE THAT I HAVE BEEN SO INFORMED AND THAT I GIVE MY CONSENT. FEDERAL REGULATIONS REQUIRE WRITTEN INFORMED CONSENT PRIOR TO PARTICIPATION IN THIS RESEARCH STUDY SO THAT I KNOW THE NATURE AND THE RISKS OF MY PARTICIPATION AND CAN DECIDE TO PARTICIPATE OR NOT PARTICIPATE IN A FREE AND INFORMED MANNER.

PURPOSE

I am being invited to voluntarily participate in the above-titled research project. The purpose of this project is to evaluate the toxicity (side effects) and safety of gene therapy of malignant melanoma by introducing a manufactured gene substance into a melanoma tumor nodule. It is hoped to induce immunity to the melanoma. However, this is a phase I study which means it is designed to test the safety of 3 different doses of the gene given 1, 2 or 3 times and has no assurance that it will have any benefit, although any effect on tumor size will be observed.

SELECTION CRITERIA

I am being invited to participate because I have metastatic malignant melanoma which means that the tumor (cancer) has spread to lymph nodes (glands) or internal organs such as liver or lung. Furthermore, my tumor is no longer or not amenable to conventional treatment with surgery, radiation or approved drugs.

Patient must be HIV negative.

If I am female I must not be pregnant or nursing. Men and women of child-bearing age and capacity must use effective birth control during study participation (condoms are highly encouraged).

A total of 15 patients whose response can be evaluated will be enrolled at the Arizona Cancer Center.

STANDARD TREATMENT(S)

There are no known cures for patients with my disease. Also, before being eligible for this protocol I will have failed on or been unable to take front line chemotherapy which has a 50% response rate. Alternative treatments available to me include the delivery of x-ray treatment to sites of local disease (50% response rate), medication to control pain (close to 100% response rate) and certain established chemotherapy treatments of experimental drugs are being evaluated at other centers to which I can be referred. These could have response rates in the range of 20% but this is not certain as they are experimental. Experimental treatments are under investigation which attempt to stimulate the immune system to reject the tumor, and I can be referred to physicians who are conducting such trials. In some cases, proteins are injected which can stimulate the immune system. I also have the option to receive no treatment at this time.

PROCEDURE

Within 14 days before I start the test injections, I will have a complete history and physical evaluation, electrocardiogram (EKG), urinalysis, blood work (complete blood count or CBC), differential and platelets, blood chemistries, pregnancy test, hepatitis test, other HIV test, special other blood tests and my tumor sites will be measured, either by direct measurement or x-ray measurement.

If I agree to participate, I will be asked to agree to the following which is described below:

If I decide to take part in this study, I will receive my injections as an outpatient. In this study, injections of manufactured genes will be offered that may help to fight this disease in other patients. Because this treatment is experimental, I may not derive any direct benefit from it. The purpose of this study is to determine a safe dose of a new treatment which will attempt to induce tumor shrinkage. Because this is new and experimental, I will be observed to determine the side effects of the therapy. I will also be monitored for the effects of this treatment on the growth of my tumor.

By using techniques in the laboratory, it is now possible to prepare large amounts of human DNA or genetic material in bacteria. This DNA will be mixed with fat bodies called lipids, and then the mixture will be transported into my tumor by needle injection. Once introduced into the tumor, the DNA produces proteins which it is hoped, based on animal studies, will stimulate tumor tissue rejection. One protein—known as HLA-B7—causes the cells which will contain it to be recognized as "foreign enemy" by the immune system. The purpose of the study is to determine whether this treatment will induce the cells of my immune system, known as lymphocytes, to attack and kill the tumor. This type of therapy which stimulates lymphocytes is called immunotherapy.

If I qualify for this study, I will have a solution containing the DNA/lipid complex injected directly into a tumor nodule. The injections will be made under sterile conditions after providing a local anesthetic (xylocaine), and areas within a single nodule will be injected. The time of treatment is approximately 30 minutes. The treatment will be given once or repeated every 2 weeks for a total of three treatments depending on which group of the protocol I am placed. Blood samples (between 1–2 ounces) will be obtained weekly or biweekly. A CT scan will be performed before initiation of treatment and once or twice in the 2-month study period. My blood lymphocytes will be tested for their ability to respond to the HLA-B7 antigen. My blood will also be tested for evidence of toxicity (side effects) from this treatment.

At different times in the protocol, tumor biopsies will be performed. This procedure involves the injection of a local anesthetic (xylocaine) under sterile conditions, followed by insertion of a needle into the tumor nodule and withdrawal of a sample of the tumor. This procedure will be performed prior to treatment and at intervals of 2 weeks up to 3 times. These tests will be in conjunction with the treatments.

I will be checked regularly for any toxicity (side effects of the drug). Clinic visits will be required approximately every week. In addition, a collection of small quantities of blood (2–6 teaspoons) for laboratory tests will be taken weekly and X-rays every 2 months and at the end of the study. The study may be terminated before that time by my decision, side effects or if medically indicated. My follow-up after study completion will be life-long. If I expire, permission for autopsy will be requested.

RISKS

All current methods of anticancer treatment (whether standard or experimental) have potential side effects. In studies thus far conducted in a few patients, no side effects have been noted. However, this is so small that the incidence of side effects for this therapy are really not known. My follow-up after study completion will be life-long.

I will be kept informed of results from this study while I am receiving the drug, especially regarding any finds which might affect my willingness to participate.

There are potential side effects and risks to this procedure. First, I may experience mild discomfort from needle injections or tumor biopsies. I may have mild discomfort and bleeding from the tumor biopsy. I will be given a local anesthetic to minimize the discomfort. Also, if the injections are into nodules in the liver, bleeding may be serious and require surgery to stop it. If the injections are in the lung area the lung may be punctured requiring hospitalization or a chest tube. There is a risk of death due to bleeding in pneumothorax. Second, even though the DNA inserted into my tumor is considered harmless to me, events could occur within normal cells that allow them to become cancerous. Laboratory studies suggest that this possibility is unlikely. However, this is a new procedure and it is not known whether cells could become abnormal after long periods of time. In animal studies, the development of cancer cells has not been observed in any animals tested. Third, the inserted DNA will contain a gene that inactivates certain antibiotics in bacteria. This protein is not likely to be made in humans, and many other antibiotics that are not inactivated will be available and effective in treating any potential bacterial infections.

It has been explained to me that this procedure, called direct gene transfer, has been used before in human patients only in one preliminary clinical trial. Because this procedure is new, it is possible that despite our extensive efforts, other unforeseen problems may arise, including the very remote possibility that death may occur.

I will undergo biopsy of tumor and other tissue, on several occasions before and after injection. Blood and tissue specimens will be taken where possible to follow the duration and effects of HLA-B7 expression. If this is successful, I will be immunized to the HLA-B7 protein. In the event that I should require an organ transplant, I would not be able to receive an organ from an individual who makes this protein, which occurs in approximately 15% of donors.

BENEFITS

This study should provide the information needed to subsequently conduct a phase II study to determine if this approach will lead to responses. The injections I will receive may induce an immune response to my tumor and a remission (meaning a shrinkage of the cancer for weeks to months).

CONFIDENTIALITY

Medical information about me and my progress in this study will remain confidential. Should the results of this study be reported or published, no individual patient names will be used. In accord with the requirements of the Food and Drug Administration (FDA), occasionally medical records of patients may be reviewed by medical monitors of sponsoring agencies whose duty it is to oversee this research to verify its accuracy. This may include the sponsoring company (Vical Inc. San Diego, California), the National Cancer Institute and the FDA.

PARTICIPATION COSTS

The experimental drug HLA-B7 gene in a plasmid liposome (fatty substance) delivery system will be supplied by the study sponsor. I will be charged for the standard tests associated with the delivery of routine medical care for my disease. This includes routine blood tests, x-rays, scans, and physician's charges. The charges associated with the management of the side effects of treatment (medication, etc.) will also be billed to me or a third party carrier. All costs are standard for medical care involving these treatments. The drug and its administration and any additional testing will be covered by the sponsor. I can obtain further information from Evan M. Hersh, MD (Principal Investigator) at (602) 626-2250 or page at 694-6000, (pager 1240), Dr. Evan Unger (602) 694-2515 (pager 2042), Dr. James Warneke (602) 626-7754 (pager 2429) or Dr. Alison Stopeck (602) 626-2816 (pager 1231).

LIABILITY

I understand that adverse reactions are possible in any research program despite the use of high standards of care and could occur through no fault of mine or the investigator involved. Reactions which can be foreseen have been described in this consent form. However, unforeseeable harm may also occur and may require care.

I understand that money for research-related side effects or harm or for wages or time lost is not available. Necessary emergency medical care directly related to this treatment will be provided from Evan H. Hersh, MD through the University of Arizona Health Sciences Center. I can obtain further information from Evan H. Hersh, MD (Principal Investigator) at (602) 626-2250, Dr. Evan Unger (602) 694-2515 (pager 2042), Dr. James Warneke (602) 626-7754 (pager 2429) or Dr. Alison Stopeck (602) 626-2816 (pager 1231). If I have questions concerning my rights as a research subject, I may call the Human Subjects Committee office at (602) 626-6721.

AUTHORIZATION

BEFORE GIVING MY CONSENT BY SIGNING THIS FORM, THE METHODS, INCONVENIENCES, RISKS AND BENEFITS HAVE BEEN EXPLAINED TO ME AND MY QUESTIONS HAVE BEEN ANSWERED. I UNDERSTAND THAT I MAY ASK QUESTIONS AT ANY TIME AND THAT I AM FREE TO WITHDRAW FROM THE PROJECT AT ANY TIME WITHOUT CAUSING BAD FEELINGS OR AFFECTING MY MEDICAL CARE. MY PARTICIPATION IN THIS PROJECT MAY BE ENDED BY THE INVESTIGATOR OR BY THE SPONSOR FOR REASONS THAT WOULD BE EXPLAINED. NEW INFORMATION DEVELOPED DURING THE COURSE OF THE STUDY WHICH MAY AFFECT MY WILLINGNESS TO CONTINUE IN THIS RESEARCH PROJECT WILL BE GIVEN TO ME AS IT BECOMES AVAILABLE. I UNDERSTAND THAT THIS CONSENT FORM WILL BE FILED IN AN AREA DESIGNATED BY THE HUMAN SUBJECTS COMMITTEE WITH ACCESS RESTRICTED TO THE PRINCIPAL INVESTIGATORS. EVAN M. HERSH, MD, EVAN UNGER, MD, JAMES WARNEKE, MD OR ALISON STOPECK, MD OR AN AUTHORIZED REPRESENTATIVE OF THE HEMATOLOGY/ONCOLOGY SECTION OF THE CANCER CENTER. I UNDERSTAND THAT I DO NOT GIVE UP ANY OF MY LEGAL RIGHTS BY SIGNING THIS FORM. A COPY OF THIS SIGNED CONSENT FORM WILL BE GIVEN TO ME.

SIGNATURES:

Subject	Date	Parent/Legal Representative	Date

I have carefully explained to the subject the nature of the above project. I hereby certify that to the best of my knowledge the person who is signing this consent form understands clearly the nature, demands, benefits and risks involved in his/her participation. A medical problem or language or educational barrier has not precluded this understanding.

Investigator	Date	Witness	Date